



UNIVERSITI PUTRA MALAYSIA

**CHARACTERIZATION OF *EPERYTHROZON OVIS* ISOLATED
FROM SHEEP AND GOATS IN MALAYSIA**

MD. ERSHADUZZAMAN

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By

MD. ERSHADUZZAMAN

**Thesis Submitted in Fulfilment of the Requirement for the Degree of Doctor of
Philosophy in the Faculty of Veterinary Medicine
Universiti Putra Malaysia.**

December 2001



DEDICATION

**TO MY PARENTS, BROTHERS, SISTERS, MY WIFE FERDOUSI BEGUM,
MY DAUGHTER JARIN TASNIM, LATE BROTHER - IN -LAW SHAMJIDUL
HAQUE AND LATE MOTHER-IN-LAW FOZILATUN NESA FOR THEIR
MORAL SUPPORT AND ENCOURAGEMENT**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

CHARACTERIZATION OF *EPERYTHROZOOM OVIS* ISOLATED FROM SHEEP AND GOATS IN MALAYSIA

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MD. ERSHADUZZAMAN

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Chairman: Associate Professor Che' Teh Fatimah Nachiar Iskandar, Ph.D.

Faculty: Veterinary Medicine

The characteristics of *Eperythrozoon ovis* isolated from sheep and goats blood were studied by several approaches. Detection of *E. ovis* from naturally infected sheep and goats was compared by light microscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), indirect immunofluorescent antibody test (IFAT) and confocal microscopy. It was concluded that the Giemsa staining is cheap, fast and easy to perform, but it may not be specific when *E. ovis* become difficult to distinguish from stain deposits or dust particles. The IFAT was rapid, specific and sensitive, but it required specific hyperimmune serum and sometimes it produced background glow that degrades the images. The confocal microscopic examination greatly enhanced images of *E. ovis* and was more sensitive than IFAT. The SEM and TEM are indispensable tools for the unambiguous identification of *E. ovis* morphology and it also provide ultrastructural detail of the organism.

In vitro culture and maintenance of *E. ovis* was successfully done upto 408 hours in tissue culture media. After intensive screening, the following conditions were found to be optimal for maintenance of red blood cell attachment by *E. ovis*: heparin as the anticoagulant for blood collection, incubation with Eagle's medium under 5% CO₂ and supplemented with inosine and foetal calf serum, and refreshment of medium every 12 hours. An attempt to propagate *E. ovis* in 8 days old embryonated chicken eggs by inoculating through the yolk sac, chorioallantoic membrane and allantoic sac was carried out. Infectivity was checked impression smears made from organs (liver, spleen and yolk sac membrane) of dead and live embryos and stained with Giemsa and further confirmed by IFAT. Among the three routes of inoculation, yolk sac was the most suitable route for propagation of *E. ovis*. Large number of *E. ovis* organisms were seen in yolk sac membrane.

Western blotting analysis of the purified sample using hyperimmune serum prepared by injecting purified *E. ovis* antigens collected from infected sheep into rabbits, revealed five protein bands with MW 180, 172, 118, 95 and 80 kDa were identified as the *E. ovis* specific bands. Among the 5 selected proteins MW 95 kDa was the most dominant. These protein were detected from infected sheep and goats indicating that the protein profiles of *E. ovis* isolated from sheep and goats were similar.

Polymerase chain reaction (PCR) of the 16S rRNA gene was investigated to determine its potential as a means of detecting *E. ovis* infection in sheep and goats. PCR produced a specific product of approximately 1500 bp from infected but not uninfected

samples. Sensitivity studies indicated that the PCR protocol was capable of amplifying total genomic *E. ovis* DNA in quantities as low as 20 ng.

In conclusion, this study discussed for the first development of PCR based assay to detect *E. ovis* from naturally infected sheep and goats. It seems that the PCR assay is specific and very sensitive compared to other test. Development of *in vitro* maintenance study provides information about the establishment of *in vitro* culture system for the maintenance and propagation of *E. ovis*. This study also indicated that the protein profiles of *E. ovis* isolated from sheep and goats were similar.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN *EPERYTHROZON OVIS* YANG DIPENCILKAN DARIPADA BIRI-BIRI DAN KAMBING DI MALAYSIA

Oleh

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Ciri-ciri *Eperythrozon ovis* yang dipencil daripada darah biri-biri dan kambing telah dikaji melalui beberapa pendekatan. Pengesanan *E. ovis* daripada biri-biri dan kambing yang terjangkit secara semulajadi telah dibandingkan menggunakan mikroskop cahaya, mikroskop elektron penapis (SEM), mikroskop elektron transmissi (TEM), ujian antibodi imunopendarfluor tak langsung (IFAT) dan mikroskop konfokal. Secara kesimpulan, pewarnaan Giemsa adalah murah, cepat dan mudah untuk dijalankan tetapi ia mungkin tidak spesifik apabila *E. ovis* sukar dikenalpasti daripada mendapah pewarna atau partikal habuk. IFAT adalah cepat, spesifik dan sensitif, tetapi ia memerlukan serum hiperimun spesifik dan kadang kala ia menghasilkan latar belakang yang mengurai imej. Ujian mikroskop konfokal sememangnya meningkatkan imej *E. ovis* dan lebih sensitif daripada IFAT. SEM dan TEM adalah alat yang perlu bagi pengecaman tidak kabur morfologi *E. ovis* dan ia juga menyedraikan butir-butir ultrastruktur bagi organisma tersebut.

Kultur *in vitro* dan pengekalan *E. ovis* telah berjaya dilakukan sehingga 408 jam di dalam medium kultur tisu. Selepas penyaringan secara intensif, keadaan berikutnya didapati optima untuk mengekalkan pelekatan sel darah merah oleh *E. ovis*; heparin sebagai antigumpal untuk pengumpulan darah, pengeraman dengan medium Eagle di bawah 5% CO₂ dan ditambah dengan inosina dan serum fetus anak (bovin), dan pertukaran medium setiap 12 jam. Satu percubaan untuk membiak *E. ovis* dalam telur ayam berembrio berumur lapang hari dengan menginokulat melalui kantung yolka, membran korioalantois dan kantung alantois telah dijalankan. Kadar jangkitan adalah tekanan lumuran yang terhasil daripada organ-organ (hati, limpa dan membran kantung yolka) yang mati dan embrio yang hidup dan diwarnakan dengan Giemsa dan seterusnya dipastikan melalui IFAT. Di antara tiga laluan penginokulan, kantung yolka merupakan laluan yang paling sesuai untuk pembiakan *E. ovis*. Sebilangan besar organisma *E. ovis* telah dilihat di dalam membran kantung yolka.

Analisis penurapan Western bagi sampel yang ditulenkan menggunakan serum hiperimun yang disediakan dengan menyuntik antigen *E. ovis* tulen yang dikumpulkan daripada biri-biri terjangkit ke dalam amab, menunjukkan lima jalur protein dengan berat molekul 180, 172, 118, 95 dan 80 kDa telah dikenalpasti sebagai jalur spesifik *E. ovis*. Di kalangan lima protein, berat molekul 95 kDa adalah paling dominan. Protein ini telah dikesan daripada biri-biri dan kambing terjangkit menunjukkan bahawa profil protein *E. ovis* yang dipencilkan daripada biri-biri dan kambing adalah serupa.

Tindak balas rantai polimerase (PCR) bagi gen 16S rRNA telah diselidiki untuk menentukan potensi gen tersebut sebagai satu cara pengesanan jangkitan *E. ovis* dalam biri-biri dan kambing. PCR menghasilkan produk spesifik kira-kira 1500bp daripada sampel terjangkit tetapi sebaliknya bagi sampel tidak terjangkit. Kajian kepekaan menunjukkan bahawa protokol PCR boleh mengamplifikasi keseluruhan genom DNA *E. ovis* dalam kuantiti serendah 20 ng.

Secara kesimpulan, kajian ini membincangkan pembangunan pertama penguji PCR untuk mengesan *E. ovis* daripada biri-biri dan kambing yang terjangkit semulajadi. Penguji PCR adalah spesifik dan sangat sensitif berbanding ujian lain. Pembangunan bagi kajian pengekalan *in vitro* menyediakan maklumat tentang penghasilan sistem kultur *in vitro* bagi pengekalan dan pembiakan *E. ovis*. Kajian ini juga menunjukkan bahawa profil protein *E. ovis* yang dipencilkan daripada biri-biri dan kambing adalah serupa.

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I certify that an Examination Committee met on 7th December 2001 to conduct the final examination of Md. Ershaduzzaman on his Doctor of Philosophy thesis entitled "Characterization of *Eperythrozoon ovis* Isolated from Sheep and Goats in Malaysia" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.



MD. ERSHADUZZAMAN

Date: December 27, 2001

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